University of British Columbia, Department of Medical Genetics

Developing an immunocompetent preclinical model to test PODO447-ADC, a tumor-specific antibody–drug conjugate.

Jiaqi Wu, Julyanne Brassard, Michael R. Hughes, Diana Canals Hernaez, Aileen Liman, Pamela Dean, Calvin Roskelley and Kelly McNagny

Abstract

Podocalyxin (PODXL) is a sialomucin member of the CD34 protein family. In normal development, PODXL is expressed at high levels in specialized glomerular epithelial cells called podocytes and can also be detected at lower levels in other cells including hematopoietic progenitor cells, endothelial cells, and platelets. Previous studies have repeatedly established PODXL as a prognostic indicator of progression and poor outcome in many forms of cancers including ovarian, pancreatic, and breast cancer. PODXL plays a key role in the formation of primary tumors as well as increasing the invasive migratory potential of tumor cells. Efforts to exploit this target have led to the further development of PODO447-ADC, an antibody-drug conjugate designed to selectively target a tumor-associated glycoform of PODXL. Treatment of PODO447-ADC in immunodeficient NSG mice implanted with patient-derived endometrial cancer shows significantly reduced tumour size and improvement of host survival. Nevertheless, the absence of the immune system removes a significant component of the body involved in cancer regulation and development and represents a potential confounding variable in the efficacy of PODO447-ADC treatment. To address these limitations, we recently developed a transgenic model of humanized-podocalyxin (Hu-PODO) mouse where the chimeric PODXL protein is expressed under the control of the mouse promoter, providing an excellent preclinical model to evaluate the potential efficacy of PODO447-ADC treatment. To evaluate the effect of a competent immune response on PODO447-ADC treatment, we have engineered mouse ID8 (ovarian) and KPCY (pancreatic) tumor cell lines to express human PODXL protein. These tumors are engrafted in immune-competent Hu-PODO mice and treated with PODO447-ADC to assess how a functional immune system interacts with PODO447-ADC treatment to effectively reduce tumor growth. In conclusion, our analysis will optimize PODO447-ADC as an effective therapeutic strategy against gynecological cancers as well as a variety of other PODO447 expressing tumors.

Background

Podocalyxin (PODXL) in the body

- Sialomucin member of the CD34 protein family
- Highly expressed in podocytes
- Regulate adhesion in tissues

PODXL in Tumor Pathology.

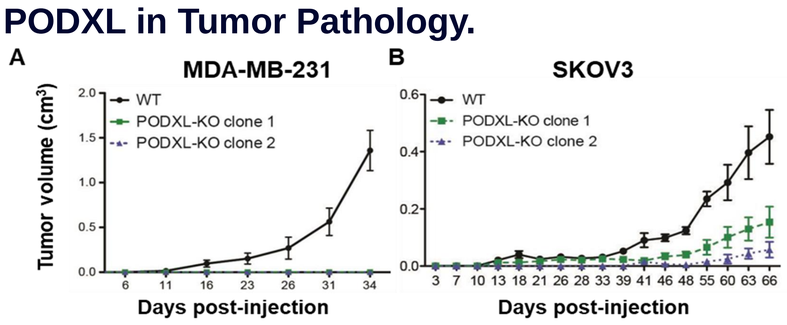


Figure 1: Podxl expression promotes primary tumor growth. Tumor growth overtime of A) MDA-MB-231 (breast cancer cell line) and B) SKOV3 (ovarian cancer cell line) WT and PODXL-KO cells ($P < 0.0001$; two-way ANOVA test). Data come from 1 experiment with 3-4 mice per group

PODO447 as a cancer therapeutic target

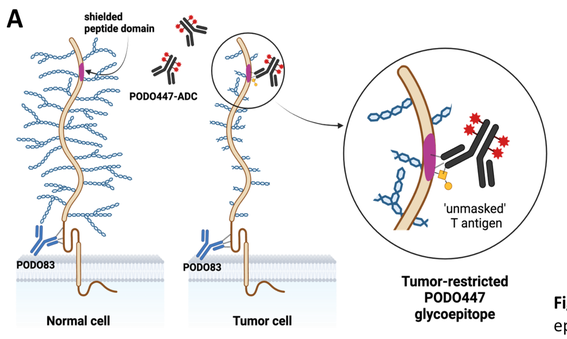


Figure 2: Schematic representation of **A)** the POOD83 and the PODO447 epitopes and **B)** the chemical structure of the PODO447-ADC. Created with Biorender.com

PODO83 antibody: binds to the core protein of podocalyxin in tumor and healthy tissues.
PODO447 antibody: recognizes a glycoepitope of podocalyxin that is exclusively expressed by some tumors, not by any healthy tissues.

- Due to its tumor specificity PODO447 is a great candidate for the development of an **antibody-drug conjugate (ADC).**

AIMS

1. Develop a patient-derived xenograft (PDX) tumor model of PODO447-expressing endometrial cancer in immunodeficient mice.
2. Test the efficacy of PODO447-ADC in treating PDX tumors.
3. Engineer mouse cancer cell lines to express human podocalyxin and PODO447

Methods & Results

Figure 3. Generation of an endometrial cancer (EC) PDX model expressing the core protein of podocalyxin and the PODO447 tumor-specific glycoepitope.

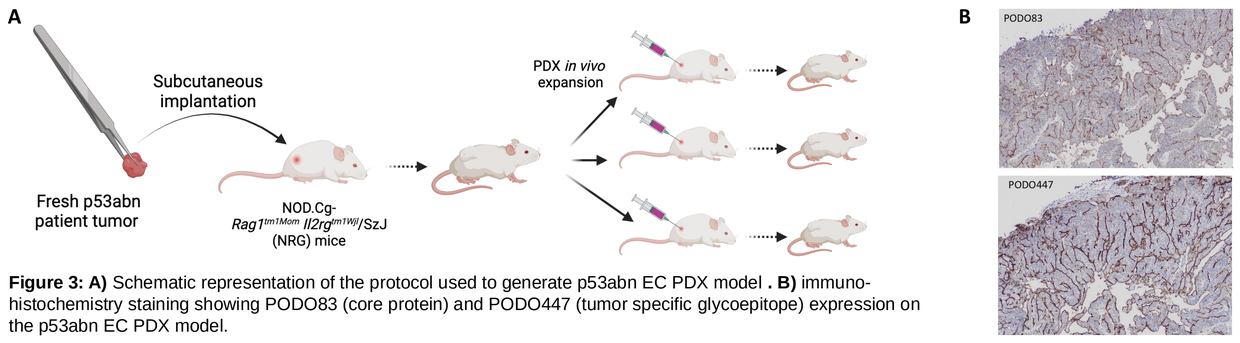
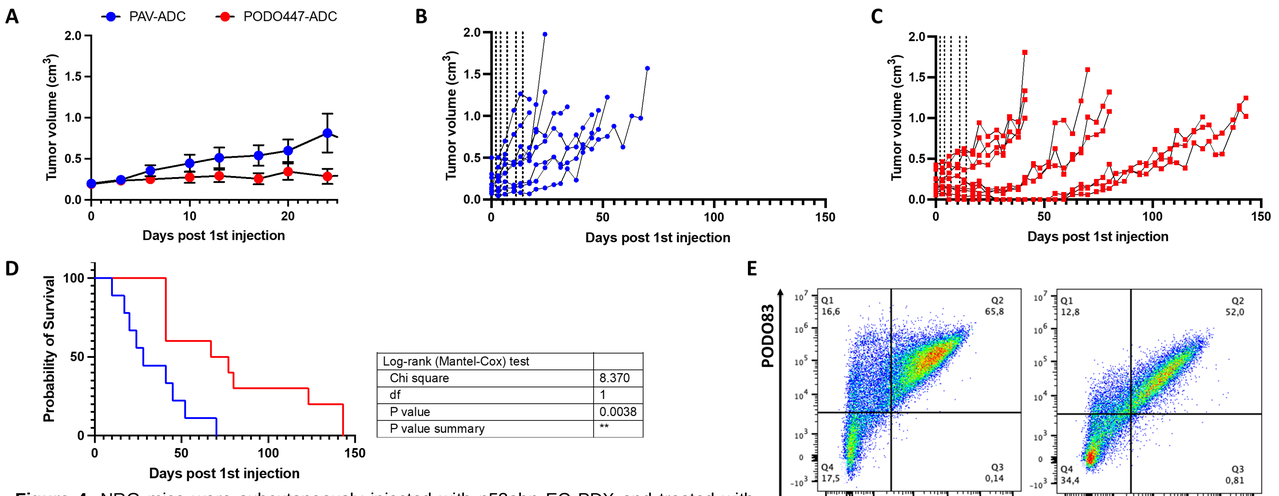


Figure 3: A) Schematic representation of the protocol used to generate p53abn EC PDX model . **B)** immuno-histochemistry staining showing PODO83 (core protein) and PODO447 (tumor specific glycoepitope) expression on the p53abn EC PDX model.

Figure 4. PODO447-ADC treatment significantly reduced p53abn EC PDX tumor growth and improved mouse survival.



Log-rank (Mantel-Cox) test	
Chi square	8.370
df	1
P value	0.0038
P value summary	**

Figure 4: NRG mice were subcutaneously injected with p53abn EC PDX and treated with control ADC (Palivizumab-MMAE) in blue or PODO447-ADC (PODO447-MMAE) in red. ADCs were injected twice a week for a total of 5 injections at a concentration of 4 mg/Kg. **A-B-C)** Tumor volume **D)** mouse survival **E)** Representative flow cytometry profile of PODO83 and PODO447 expression on relapsing PODO447-ADC treated tumors.

Figure 5. Cell engineering process of mouse cancer cell lines to express human podocalyxin

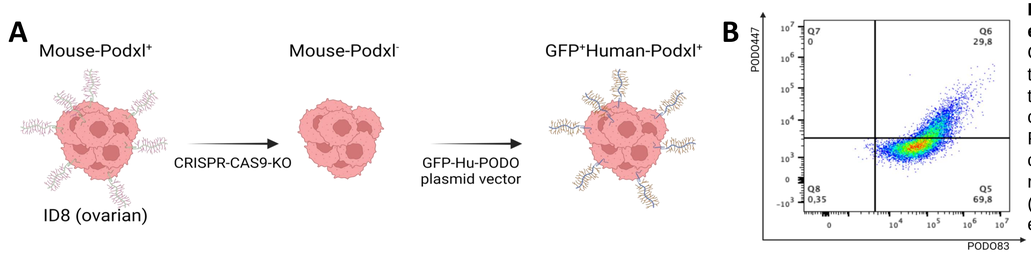


Figure 5: ID8 cell engineering process. A) Graphical representation of the engineering process used to generate mouse cancer cell lines that express human Podxl protein. B) Flow cytometry plots representing human Podxl (PODO83) and PODO447 expression on ID8 cells.

Figure 6. PODO447-ADC treatment does not significantly reduce tumor growth or improve mouse survival.

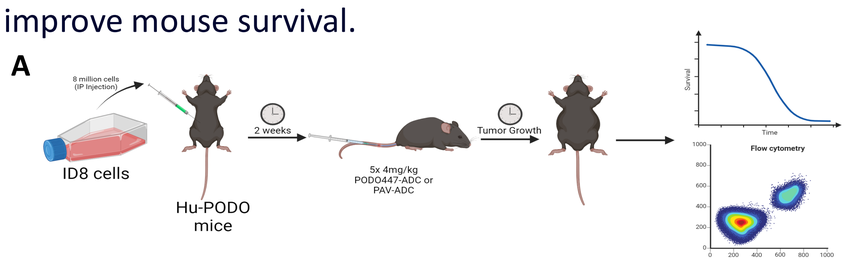
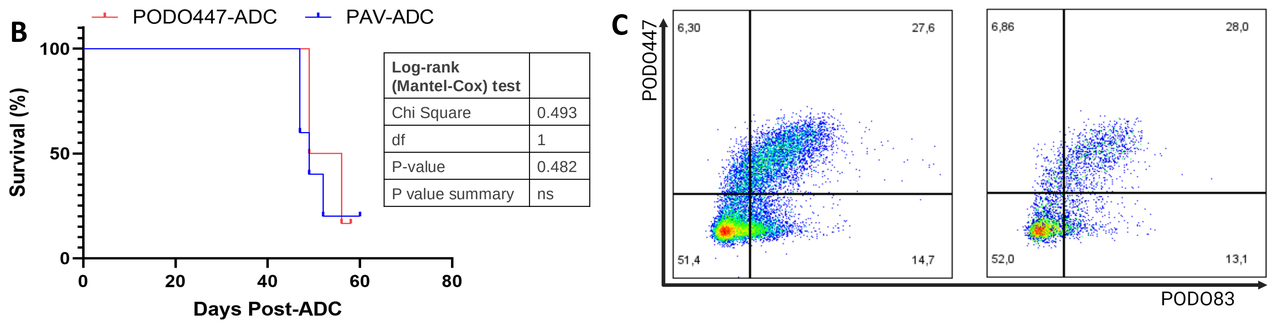


Figure 6: Hu-PODO mice were intraperitoneally injected with 8 million human podocalyxin expressing ID8 cancer cells and treated with control ADC (Palivizumab-MMAE) in blue or PODO447-ADC (PODO447-MMAE) in red. ADCs were injected twice a week for a total of 5 injections at a concentration of 4 mg/Kg. **A)** Graphical representation of the treatment process. **B)** mouse survival **C)** Representative flow cytometry profile of PODO83 and PODO447 expression on relapsing PODO447-ADC treated tumors.



Log-rank (Mantel-Cox) test	
Chi Square	0.493
df	1
P-value	0.482
P value summary	ns

Conclusions and Future Directions

Key Takeaways

1. PODO447-ADC can effectively treat human EC in immunocompromised mouse models
2. Engineered ID8 mouse ovarian cancer cells can express human podocalyxin and PODO447.

Next Steps

Test the efficacy of the PODO447-ADC in EC PDX models derived from other patient tumors.

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RENDER
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